Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) *Bos taurus* cattle during controlled heat challenge[1]

B. Scharf,[*][2] J. A. Carroll,[†] D. G. Riley,[‡] C. C. Chase Jr.,[‡] S. W. Coleman,[‡]
D. H. Keisler,[*] R. L. Weaber,[*] and D. E. Spiers[*]

*Division of Animal Science, University of Missouri, Columbia; †USDA-ARS, Livestock Issues Research Unit, Lubbock, TX; and ‡USDA-ARS, Subtropical Agricultural Research Station, Brooksville, FL

ABSTRACT: A study was performed to evaluate differences in thermoregulatory ability of 2 *Bos taurus* breeds with known differences in heat tolerance. Nine Angus (AG; 304 ± 7 kg of BW) and 9 Romosinuano (RO; 285 ± 7.5 kg of BW) steers were transported to the Brody Environmental Center at the University of Missouri. Steers were housed for 18 d at thermoneutrality (TN; 21°C) before initiation of heat stress (HS), which consisted of daily cyclic air temperature (26°C, night; 36°C, day) for 14 d. Rectal temperature and respiration rate were measured 6 times daily throughout the study. Sweat rates at shaved skin sites were recorded on specific days. Blood samples were taken once per week. Angus steers maintained rectal temperature 0.5°C greater than RO at TN ($P < 0.001$). Likewise, respiration and sweat rates were greater ($P < 0.001$) in AG than RO at TN ($P < 0.05$). Rectal temperature increased during HS for both breeds with AG maintaining greater temperatures ($P < 0.001$). Both breeds increased respiration rate during HS, with AG steers exhibiting the greater rate ($P < 0.001$). Sweat rate increased more than 4-fold during HS ($P < 0.001$), followed by reduction after 7 d. Even after HS acclimation, AG exhibited the greater sweat rate ($P < 0.001$). Breed differences for serum leptin, creatinine, and cholesterol were found throughout the study with AG being greater than RO. Although there were no breed differences ($P = 0.21$) at TN, only AG steers exhibited a HS-induced increase ($P < 0.05$) in prolactin, creatinine, and cholesterol concentrations to suggest that an increase in rectal temperature is required for this effect. Use of rectal temperature along with endocrine markers, such as prolactin, may aid in the identification of *B. taurus* sensitivity to heat.

Key words: cattle, heat tolerance, physiological marker

INTRODUCTION

Development of a breed of cattle that can tolerate heat stress (**HS**) and maintain productivity has been a long-term goal of researchers and cattle breeders. Historically, most research in this area has emphasized comparisons of heat-tolerant *Bos indicus* cattle (e.g., Brahman) vs. heat-intolerant *Bos taurus* cattle (Brody, 1956; Finch et al., 1982; Gaughan et al., 1999; Beatty et al., 2006). However, isolation of specific differences in thermoregulatory ability is challenging due to the

many physical and genetic differences that could have secondary influences.

Current interest has shifted toward using breeds of *B. taurus* cattle from tropical climates that might be heat tolerant compared with Angus (**AG**) and superior to *B. indicus* cattle in terms of reproduction, growth, and carcass quality (Spiers et al., 1994; Chase et al., 1997). The Romosinuano (**RO**) is a tropically adapted Criollo beef breed that is native to Colombia, South America (Chase et al., 1997). Previous research suggests that RO exhibit heat tolerance, as well as superior growth and fertility on tropical pastures compared with AG cattle (Vogt et al., 1991). Likewise, they are noted for longevity and docile temperament (Chase et al., 1997; Elzo et al., 1998).

There have been few studies to compare the specific thermoregulatory abilities of RO under laboratory or field conditions (Vogt et al., 1991; Spiers et al., 1994; Hammond et al., 1996). The purpose of this study was

Scharf et al.

to determine the thermoregulatory responses of heat-tolerant RO and heat-sensitive AG *B. taurus* breeds to a controlled heat challenge in order to derive potential physiological and biochemical indicators of heat tolerance.

MATERIALS AND METHODS

The experimental protocol and procedures were approved by the University of Missouri Animal Care and Use Committee.

Animals

Nine AG (304 ± 7.0 kg of BW) and 9 RO steers (285 ± 7.5 kg of BW) were obtained from the Subtropical Agricultural Research Station (USDA-ARS) in Brooksville, Florida. Romosinuano steers were derived from embryos imported from an upgraded herd at the Centro Agronomico Tropical de Investigacion y Ensenanza in Turrialba, Costa Rica (1991 and 1993) or from purebred RO herds in Venezuela (1997). Embryo calves were born in 1991, 1993, or 1997, forming the present-day herd in Brooksville (Riley et al., 2007). Animals in the current study were raised as part of the Brooksville herd before shipment to Oklahoma and finally being brought to the University of Missouri in late January. Steers were housed in covered feedlots at the University of Missouri South Farm for training from January to March before entering the Brody Environmental Center. Steers were approximately 12 mo old at the start of the experiment. Three environmental chambers within the Brody Environmental Center were used. Each was divided into 6 stanchions for maintenance of 6 steers (3 RO and 3 AG per room). Animals were limit fed a high-concentrate diet (39% each of corn and soybean hulls, 20% dried corn distillers grains, and 2% mineral supplement; as-fed basis) at 1.6% of BW per day (AG: 5 kg; RO: 4.5 kg), with water available for ad libitum consumption.

General Procedure

Steers were housed for 18 d at thermoneutrality (**TN**; 19 to 21°C) before initiation of HS to allow acclimation to the chamber environment (acclimation is defined as "adaptive changes that occur within an organism in response to experimentally induced changes in particular climatic factors such as ambient temperature in a controlled environment"; Bligh and Johnson, 1973). Heat stress consisted of daily cyclic air temperature (26°C night: 36°C day) for 14 d (Figure 1). Transition from TN to cyclic HS conditions was accomplished over a 2-d period (d 1: 28°C; d 2: 33°C; Figure 1). Chambers at TN had a set point of 20°C and only slight fluctuations. Chambers during HS cycle increased as a step-up function with 3 set points throughout the rise phase,

followed by a 4-h stable period (36°C; 1200 to 1600 h). The decline phase consisted of 2 set points to reach the stable low temperature (26°C; 0000 to 0600 h). Environmental conditions were measured using Hobo H8 data loggers (Onset, Bourne, MA) to record air temperature (T_a) and percentage relative humidity (**RH**) every 10 min. Relative humidity was maintained under 50% during the entire study (TN: 40 to 50%; HS: 35 to 45%). Animal measurements, including respiration rate (**RR**), skin temperature (T_{skin}), and rectal temperature (T_{re}), were taken 6 times daily (0600, 1100, 1300, 1600, 1900, and 2100 h). Determination of RR was made by counting flank movement over a 1-min interval and is presented in breaths per minute (**bpm**). Skin temperatures, at 5 different shaved sites (ear, shoulder, rump, tail head, and lower tail), were measured using an infrared thermometer (model C-1600M, Linear Laboratories, Fremont, CA; accuracy ± 0.1°C). Readings were taken less than 31 cm away from each skin site with a thermometer target ratio of 3:1. Rectal temperature was measured using a thermistor thermometer (model 8110–20, Cole-Parmer Instruments, Chicago, IL). This was accomplished by inserting a YSI probe (model 400, YSI Inc., Yellow Springs, OH; accuracy: ± 0.1°C) approximately 15 cm into the rectum for 2 min. Sweat rate was measured on specific days throughout the study (targeting TN, early heat, and late heat periods) at shaved shoulder and rump sites using a calibrated, digital moisture sensor (Vapometer; Delfin Technologies Ltd., Kuopio, Finland) that determines transepidermal water loss. All calibrations are certified and performed at the company laboratory using 3 different relative humidities. The Vapometer uses a closed system approach, free of ambient airflow, to measure ambient relative humidity and temperature. This is automatically performed by the instrument before skin application (open surface area of 1.0 cm^2). The device is then held on the skin for 10 to 20 s before the evaporation rate is displayed in g/(m^2·h) (accuracy = ±10%). The time between measurements is automatically controlled to allow the relative humidity in the chamber to return to the ambient baseline measured before skin contact. Only after this baseline is achieved will the Vapometer allow the next measurement. Other recent studies have used the same type of device to measure moisture loss in a variety of environments (Nuutinen et al., 2003; Gebremedhin et al., 2007; Scharf et al., 2008a).

Blood (20 mL) was collected at 0900 h on d 16 (TN) and 31 (HS) via jugular venous puncture. Samples were collected into two 15-mL tubes and allowed to clot before centrifugation. Serum was separated by centrifugation (2,300 × *g* for 25 min; 4°C) before being removed and stored at −20°C for later analysis. Serum analyses used standard procedures. Most serum measurements were components of a larger biochemical profile produced by the Veterinary Medical Diagnostic Laboratory, University of Missouri–Columbia using an autoanalyzer (Olympus AV400, Olympus America Inc.,
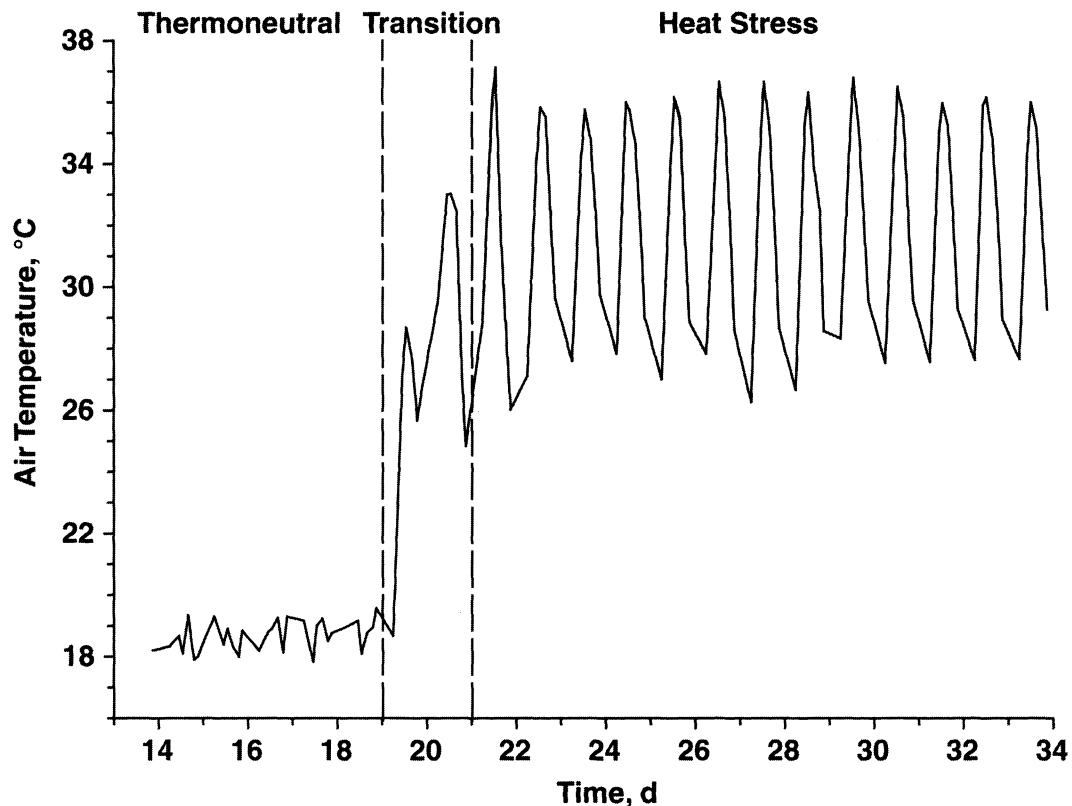
Figure 1. Mean room air temperature beginning on d 14 at thermoneutrality (19 to 21°C) and continuing through d 33, which was the last day of heat stress (night: 26°C; day: 36°C). Values were collected hourly for each day.

Melville, NY). These include albumin, chloride, cholesterol, creatinine phosphokinase, creatinine, globulin, glucose, magnesium, potassium, sodium, total protein, triglyceride, and urea nitrogen. Serum leptin concentrations were determined by a sensitive ovine leptin RIA validated for bovine serum (Delavaud et al., 2000). Standards were assayed in quadruplicate and samples in triplicate 200-μL volumes. Assay sensitivity and intraassay CV were 0.03 nmol/L and 3.1%. Serum concentrations of prolactin were determined by RIA procedures previously validated at the University of Missouri (Lutz et al., 1991). Minimum detectable concentration of prolactin in serum was 1.2 ng/tube. Intraassay CV was 9.2%.

Statistical Analyses

Data was analyzed using a repeated measures ANOVA procedure in JMP statistical software (SAS Inst. Inc., Cary, NC). All evaluations at TN were performed using the last 5 d (i.e., d 14, 15, 16, 17, and 18) before the increase in T_a. The analysis included RR, T_{skin}, sweat rate, or T_{re} as the dependent variable. Skin temperatures were included as an average of all skin sites (T_{skin}), an average of the shoulder and rump sites (T_{trunk}), or an average of ear, tail head, and lower tail ($T_{appendage}$). Breed, time, and breed × time were set as fixed effects, with animal nested within breed as a random effect. For ANOVA analyses present in text, experiment-wise

type I error rate was controlled to $\alpha = 0.05$ utilizing the Tukey-Kramer honestly significant difference adjustment procedures for multiple mean comparisons. For plotting purposes, Fisher's least significant difference (Steele and Torrie, 1980) was calculated utilizing the LSMEANS statement in PROC MIXED of SAS and is presented in the upper left portion of figures. Simple linear and polynomial regression procedures of JMP were utilized to establish relationships between animal variables (RR, T_{skin}, sweat rate, and T_{re}) and T_a. Regression coefficients for slope and model r, as well as P-values for the hypothesis test that the regression coefficients are significantly different from zero, are reported. Similarly, simple linear regressions were constructed to determine the relationship for early heat (d 21 to 24) and late heat exposure periods (d 29 to 32) using the regression procedures of JMP. Blood analyses were performed using the repeated measures ANOVA procedure in JMP with fixed and random effects as described above.

RESULTS

Steers were brought into the chambers with AG starting BW slightly larger than that of the RO (AG: 317.5 ± 7.2 kg of BW; RO: 288.0 ± 7.2 kg of BW; $P < 0.05$). While present in the chambers, animals were limit-fed at 1.6% BW daily, resulting in a slight drop in BW for both breeds over the study (AG: −13.2 ± 4.2 kg; RO:
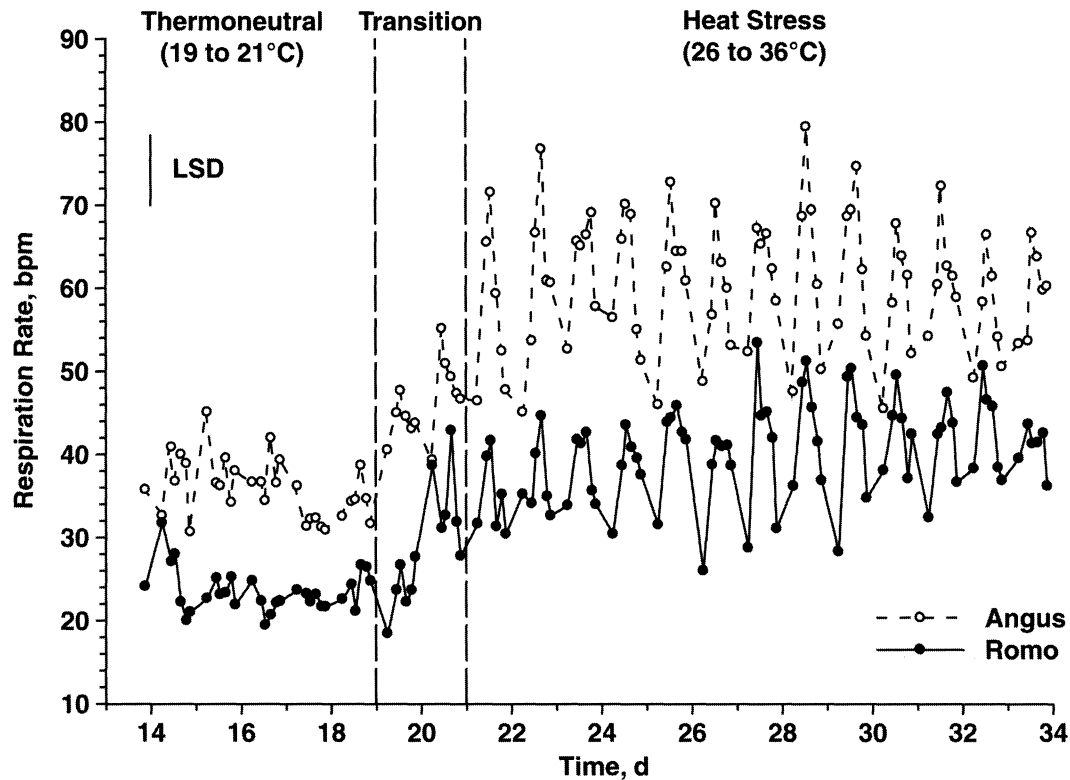
Figure 2. Mean respiration rate of Angus and Romosinuano (Romo) steers is shown as a function of time in days for the last 5 d at thermoneutrality followed by 15 d of heat stress exposure. All of the 6 sample times during a day are shown. The vertical dashed line separates thermoneutral and heat stress periods, and the small solid line is the LSD distance at $P < 0.05$. bpm = breaths per minute.

−4.6 ± 4.2 kg). Final BW at the end of the study did not differ between groups (AG: 304 ± 7.0 kg of BW; RO: 285 ± 7.5 kg of BW; $P = 0.07$).

TN

Respiration rate at TN was greater ($P < 0.001$) in AG than RO by ~12 bpm (Figure 2). There was a breed × day interaction ($P < 0.01$) but no breed × day × hour interaction ($P = 0.08$) at TN, with the RO showing no change and the AG slowly reducing RR over time (38 to 33 bpm) from d 14 to 18. After acclimation, AG steers continued to exhibit the greatest RR ($P < 0.001$). Angus steers also maintained a greater T_{re} (~0.21°C) than RO at TN ($P < 0.01$; Figure 3); however, no acclimation took place with T_{re} remaining constant over the last 3 d at TN ($P > 0.05$). There was a daily increase in T_{re} of approximately 0.25°C ($P < 0.001$) from 1100 to 1900 h at TN. Regional skin temperatures were less ($P < 0.01$) for RO than for AG steers, with the differences being 1.0°C at the extremities (Figure 4A) and 0.7°C at the trunk (Figure 4B). There was also a daily increase in skin temperature of both regions of approximately 0.7°C from 1300 to 2100 h ($P < 0.001$). The thermal circulation index was calculated using the equation $[(T_{skin} - T_a)/(T_{re} - T_{skin})$; Burton and Edholm, 1955] as an indicator of blood flow and heat transfer to the skin. Angus steers had greater values for appendage and trunk skin sites in compari-

son with RO steers (appendage: AG = 0.91, RO = 0.77, $P = 0.036$; trunk: AG = 1.15, RO = 1.0, $P < 0.02$). It is apparent that the thermoneutral temperature used in this study represents a cooler T_a for RO steers compared with AG steers, with a reduction in blood and heat flows to the periphery. Similar results were found for sweat rates with AG being ~10 g/m²·h greater that RO (Figure 5; $P < 0.05$). All variables (T_{re}, T_{skin}, and sweat rate) were stable with the exception of RR, which stabilized within 5 d at TN, resulting in a steady baseline before heat exposure.

HS

The transition from TN to HS environments produced a more rapid increase in RR for AG compared with RO, with a 9.7 bpm increase in AG steers on the first HS day ($P = 0.05$; Figure 2) and no increase for RO steers. By the second HS day, RR of AG and RO steers had increased 13.7 and 9.9 bpm, respectively, from the last TN day ($P = 0.05$). Both breeds had a RR that remained greater than the TN level during the first week of HS, with AG steers exhibiting the greater rate (60 bpm, AG; 38 bpm, RO; $P < 0.001$; Figure 2). Respiration rate showed no acclimation ($P = 0.07$) for AG, but increased 8.4 bpm for RO from d 21 to 28. The breed differences in RR persisted into wk 2 (61 bpm, AG; 42 bpm, RO; Figure 2). During HS, there was a daily increase in RR of 15.4 bpm from 0600 to 1300 h
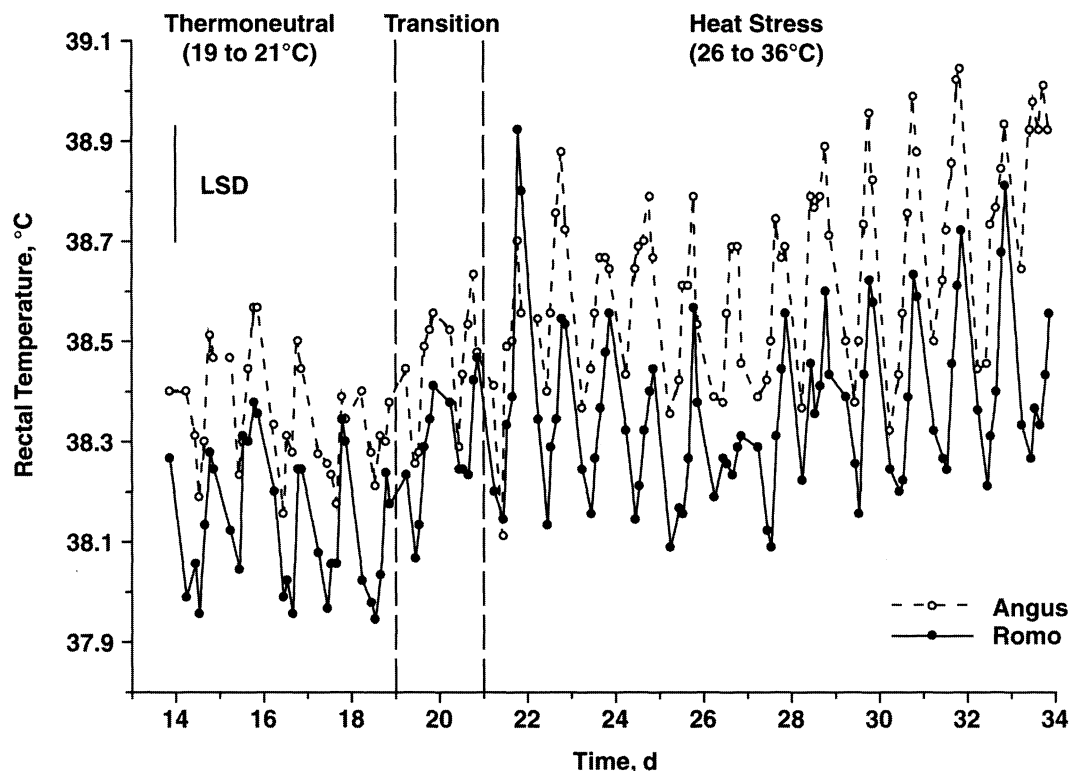
Figure 3. Mean rectal temperature of Angus and Romosinuano (Romo) steers is shown as a function of time in days for the last 5 d at thermoneutrality followed by 15 d of heat stress exposure. All of the 6 sample times during a day are shown. The vertical dashed line separates thermoneutral and heat stress periods, and the small solid line is the LSD distance at $P < 0.05$.

$(P = 0.05)$, with the increase in AG (19.3 bpm) being greater $(P < 0.05)$ than for RO (11.5 bpm).

Both breeds exhibited an increase in T_{re} during transition TN to HS (0.17°C; $P = 0.05$; Figure 3). The daily increase in T_{re} during the transition period was significant $(P < 0.01)$ from 1100 h (38.18°C) to 1600 h (38.34°C) to 1900 h (38.52°C), with no breed differences. Interestingly, it was RO steers that showed the larger increase, rising to the same T_{re} level of AG animals during the first full day of heat (d 21). However, RO steers quickly acclimated, maintaining a 0.30°C less average daily T_{re} than AG steers throughout wk 1 of HS $(P < 0.05$; Figure 3; d 21 to 27). Angus steers displayed a more delayed T_{re} response with no significant increase $(P = 0.50)$ until second day of full the heat (0.33°C; d 22). Rectal temperature during HS increased $(P < 0.05)$ by 0.10°C for each period from 1100 h (38.35°C) to 1300 to 1600 h, with a peak at 1900 h (38.70°C). Less daily T_{re} at 1100 h was different $(P < 0.05)$ for RO (38.21°C) and AG (38.49°C). The breed difference in T_{re} of 0.28°C coincided with peak daily T_{re}, which occurred at 1900 h. Rectal temperature of AG steers continued to increase during wk 2 of HS, whereas T_{re} for RO remained constant $(P < 0.01)$. This occurred despite the fact that RR remained the same for this breed.

The breed differences in T_{skin} that were observed at TN were not observed $(P = 0.13)$ during the transition from TN to HS environments. There were daily increases $(P < 0.05)$ in T_{skin} of trunk and appendage sites, be-

ginning on the first day of T_a increase and continuing to full heat exposure (Figures 4A and 5B). The increases in trunk and appendage skin sites over this period were 4.91 and 6.26°C. Average daily skin temperature in both regions for both breeds increased $(P < 0.05)$ from the first day of full heat to d 4 when peak skin temperature was observed. The increase for the trunk sites was 1.69 to 35.39°C followed by a 0.78°C reduction $(P < 0.05)$ to 34.61°C on the last day (Figure 4B). Likewise, the appendage sites increased 1.63 to 34.98°C followed by a 0.63°C reduction $(P < 0.05)$ to 34.35°C at the end of the study (Figure 4A). This reduction in T_{skin} was not due to a change in T_a because it decreased only 0.14°C from the day of peak T_{skin} to the last day of the study. Average skin temperature closely followed the cyclic T_a pattern among all skin sites. Daily increases in T_{skin} for trunk and appendage sites over the entire HS period were 3.00 and 3.25°C, respectively, from 0600 to 1300 h $(P < 0.05$; Figures 4A and 4B).

Both breeds initially increased shoulder sweat rate (4-fold) during HS but with a greater increase in AG compared with the RO (292.6 vs. 175.23 g/m²h, respectively; $P < 0.001$; Figure 5A). This was followed by a reduction after 7 d for both breeds $(P < 0.001$; Figure 5A) to approximately one-half the level of the first 3 d of HS. However, AG retained a greater sweat rate even after the acclimation period compared with the RO (200.5 vs. 110.3 g/m²·h, respectively; $P < 0.001$). Reduction in sweat rate coincided with the increase in
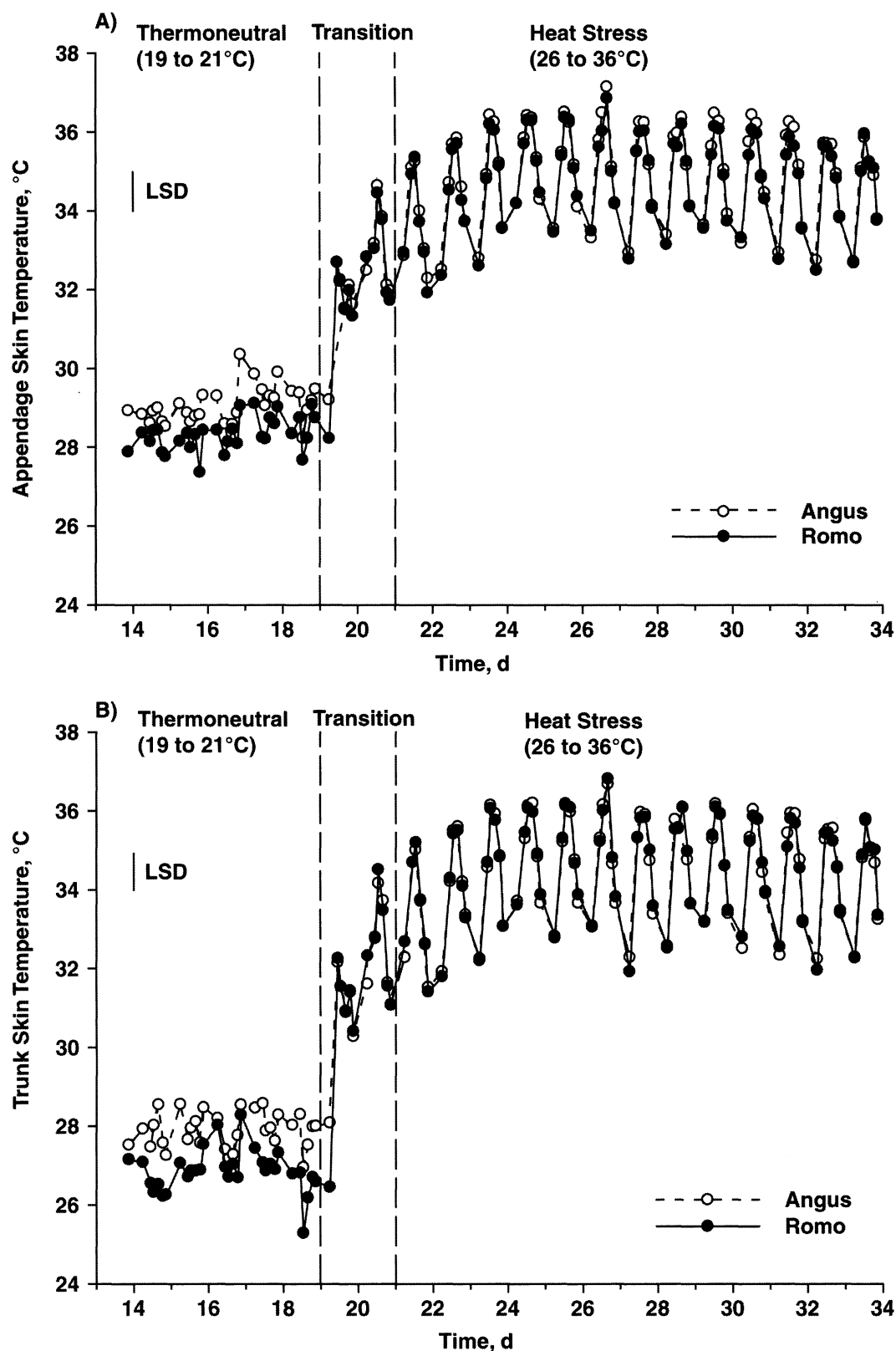
Figure 4. Mean appendage (A) and trunk (B) skin temperatures of Angus and Romosinuano (Romo) steers are shown as a function of time in days for the last 5 d at thermoneutrality followed by 15 d of heat stress exposure. All of the 6 sample times during a day are shown. The vertical dashed line separates thermoneutral and heat stress periods, and the small solid line is the LSD distance at $P < 0.05$.

T_{re} that was noted for AG cattle. Romosinuano cattle also maintained a reduced sweat rate after acclimation, but unexpectedly T_{re} showed no increase. Sweat rate was greater ($P < 0.001$) at the shoulder vs. the rump

regions during HS for both breeds (Figure 5B). However, sweat rates of shoulder and rump regions for both breeds paralleled each other during wk 1 of HS, with AG having the greater rate.

Temperature Relationships

All thermal variables were evaluated to determine the linear correlation coefficients and predictors of the thermoneutral and early HS responses, as well as the HS response alone for the 2 breeds using individual animal values. Use of TN, transition, and early HS response allowed for the determination of thermal responsiveness in the absence of acclimation (Table 1). Both RR and T_{re} are reliable and are often used as indicators of thermal status. Table 1 illustrates that all indicators of thermal input (i.e., T_a, T_{skin}, T_{trunk}, and
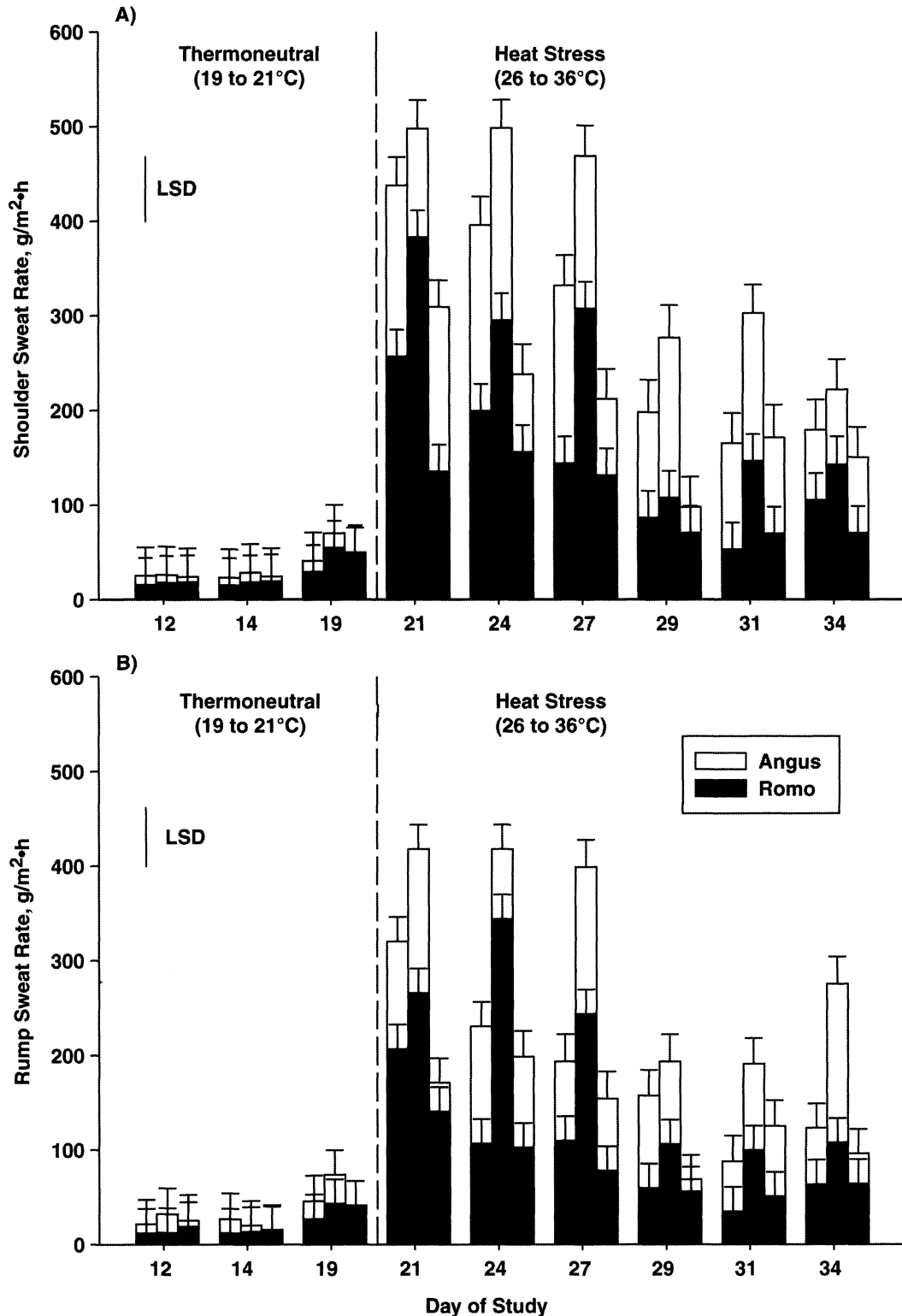

Figure 5. Mean skin sweat rate of shaved shoulder (A) and rump (B) skin sites for Angus and Romosinuano (Romo) cattle on selected days before heat exposure (i.e., pre d 20) and during heat stress (i.e., post d 20). The vertical line on top of each column is +1 SE, and the vertical LSD line is for $P < 0.05$.

Table 1. Linear regressions of variables from d 14 to 24 using individual values[1]

Variable					
Dependent	Independent	Breed	Correlation coefficient	Slope	P-value
RR	T_a	AG	0.73	1.74	0.001
		RO	0.60	0.96	0.001
RR	T_{skin}	AG	0.74	3.92	0.001
		RO	0.61	1.91	0.001
RR	T_{trunk}	AG	0.73	4.14	0.001
		RO	0.61	2.11	0.001
RR	$T_{appendage}$	AG	0.74	3.73	0.001
		RO	0.60	1.76	0.001
T_{skin}	T_a	AG	0.93	0.42	0.001
		RO	0.93	0.48	0.001
T_{re}	T_a	AG	0.33	0.01	0.001
		RO	0.22	0.01	0.050
T_{re}	T_{skin}	AG	0.35	0.03	0.001
		RO	0.27	0.03	0.010
T_{re}	T_{trunk}	AG	0.35	0.03	0.001
		RO	0.26	0.03	0.010
T_{re}	$T_{appendage}$	AG	0.34	0.03	0.001
		RO	0.28	0.03	0.010
T_{trunk}	$T_{appendage}$	AG	0.98	0.87	0.001
		RO	0.97	0.81	0.001

[1]Each incorporates both thermoneutral [air temperature (T_a): 19 to 21°C] and early heat stress (T_a: 26 to 36°C) responses into the analyses before heat acclimation. RR = respiration rate; T_{skin} = skin temperature; T_{re} = rectal temperature; T_{trunk} = average of the shoulder and rump sites; $T_{appendage}$ = average of ear, tail head, and lower tail. AG = Angus; RO = Romosinuano.

$T_{appendage}$) are reasonable predictors. In every case, the correlation coefficients were greater for AG compared with RO steers. More importantly, the responsiveness of AG steers during this transition period, as indicated by slope, was twice that of RO steers. The determinants of T_{re} again showed a greater ($P < 0.05$) correlation for AG compared with RO animals (Table 1). However, the slopes of the responses across breeds were identical and extremely small when compared with the RR responses. Both the correlation coefficients and slopes were small for the T_{re} response compared with the RR response. As expected, the relationships between T_{skin} and T_a, and T_{trunk} and $T_{appendage}$ were very large and not different ($P = 0.81$) across breeds (Table 1).

Analysis of the thermal relationships during the stable HS period (i.e., d 21 to 33) revealed that the different skin temperatures were highly correlated with each other (r = 0.90 to 0.96) and with T_a (r = 0.74 to 0.80) when data from both breeds were combined. There was little improvement when the analysis was performed on data from each breed separately. Respiration rate (r = 0.37) also showed linear correlation ($P < 0.001$) with T_a using both breeds together. However, there was a breed difference when predictors of RR were analyzed separately. Prediction of RR for AG using T_a and each skin temperature was r = 0.54 and r = 0.46 to 0.51, respectively. In contrast, the same predictor relationships for T_a (r = 0.39) and skin temperatures (r = 0.38 to 0.39) were smaller ($P < 0.05$) for RO. This was expected because daily change in RR for RO was less than for AG. Rectal temperature showed a very poor correlation

with T_a (r = −0.0002) and skin temperatures (r = 0.12 to 0.19) when both breeds were combined. Romosinuano steers regulated T_{re} almost independent of T_a and skin temperature.

Sweat rates were highly correlated with T_a during initial 3 d of HS, with AG steers having the slightly greater correlation for shoulder (r = 0.68 vs. 0.65; Figure 6) and rump (r = 0.68 vs. 0.59, data not shown) sites using a second-order polynomial regression fit. A comparison of sweat rate across skin sites was also performed using skin temperature at the site as the thermal input, to determine if this is a better input for sudomotor activity than T_a. Once again, AG steers exhibited the greater correlation ($P < 0.01$) for shoulder (r = 0.69 vs. 0.63; Figure 6) and rump (r = 0.66 vs. 0.53, data not shown) sites. Using these correlations, it was evident that use of skin temperature did not improve sweat rate prediction and that AG steers were more responsive to early HS exposure.

Thermoregulatory responses between the end of wk 1 (d 21 to 24) and end of wk 2 (d 29 to 32) of HS were compared with assess short-term acclimation to heat (Figure 7A to 7D). Linear regression was used to determine the change in response of sweat rate, RR, and T_{re} vs. T_a between the 2 wk. Comparison of the 2 breeds shoulder sweat rate during wk 1 of HS shows that, although starting at different levels, they exhibit a similar response to HS (i.e., both lines have a slope of 26.19; Figure 7C and 7D). As stated previously, both breeds showed a reduction in sweating rate after 7 d of HS. Comparing wk 1 vs. 2 of HS, all steers showed ac-

climation with a reduction ($P < 0.05$) in slope (AG = 26.19 to 17.02; RO = 26.19 to 8.98) of shoulder sweat rate vs. T_a (Figure 7C). Although AG maintained the greater rate, the breeds converged as T_a fell below 25°C. Rump sweat rate exhibited the same response as shoulder to a lesser degree (Figure 7D). Angus also maintained a greater sweat rate over RO during wk 1 and 2 of HS. Unlike sweat rate, RR showed little acclimation between weeks (AG slope = 1.92 to 1.13; RO slope = 0.65 to 0.65; Figure 7A) with AG maintaining
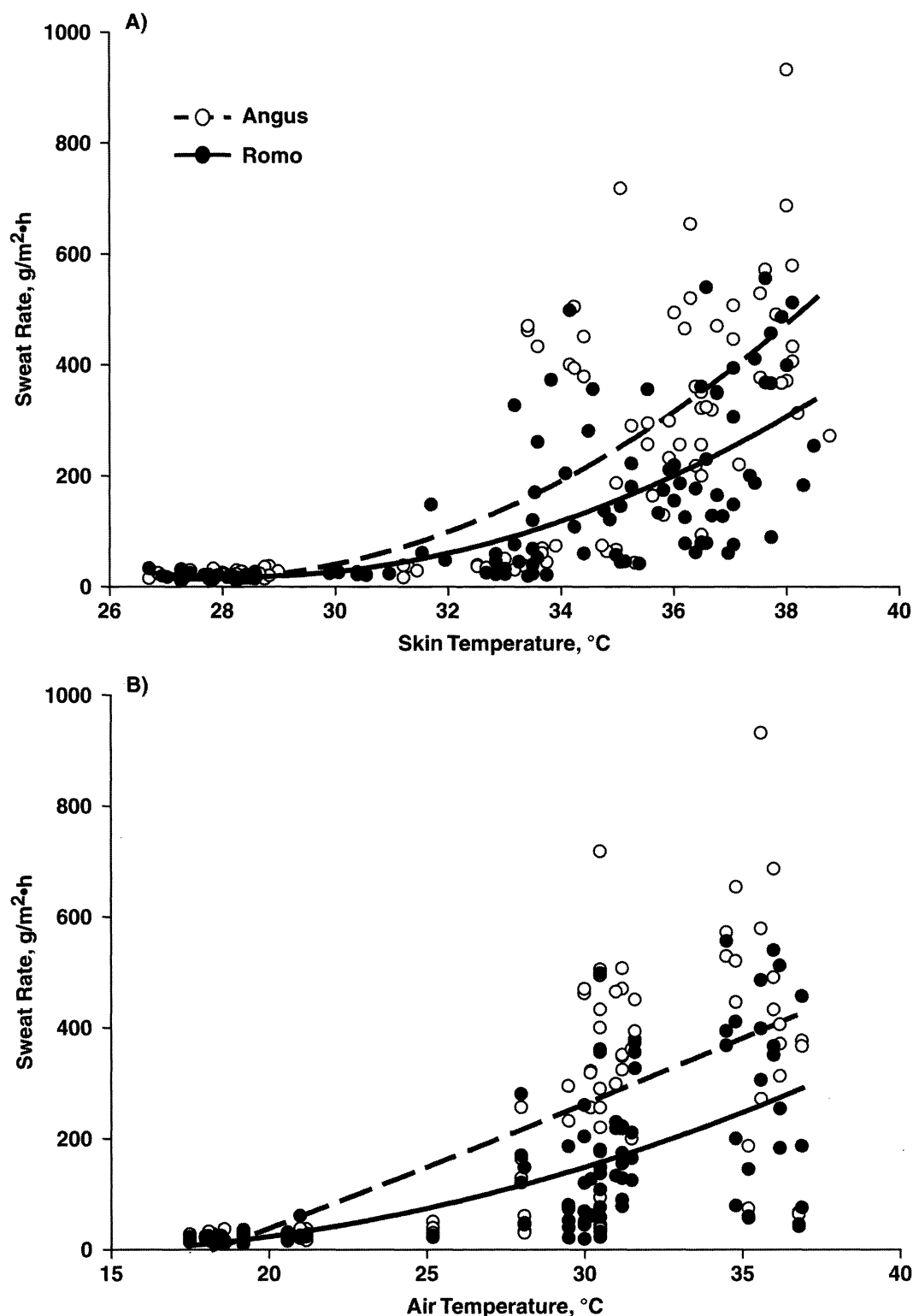

Figure 6. Shoulder sweat rate using mean group values for breed [i.e., Angus and Romosinuano (Romo)], day, and time of day for d 14 and 19 (preheat; T_a: 19 to 21°C), and d 21 and 24 (early heat; T_a: 26 to 36°C). T_a = air temperature. The fitted line is a second-order polynomial regression.
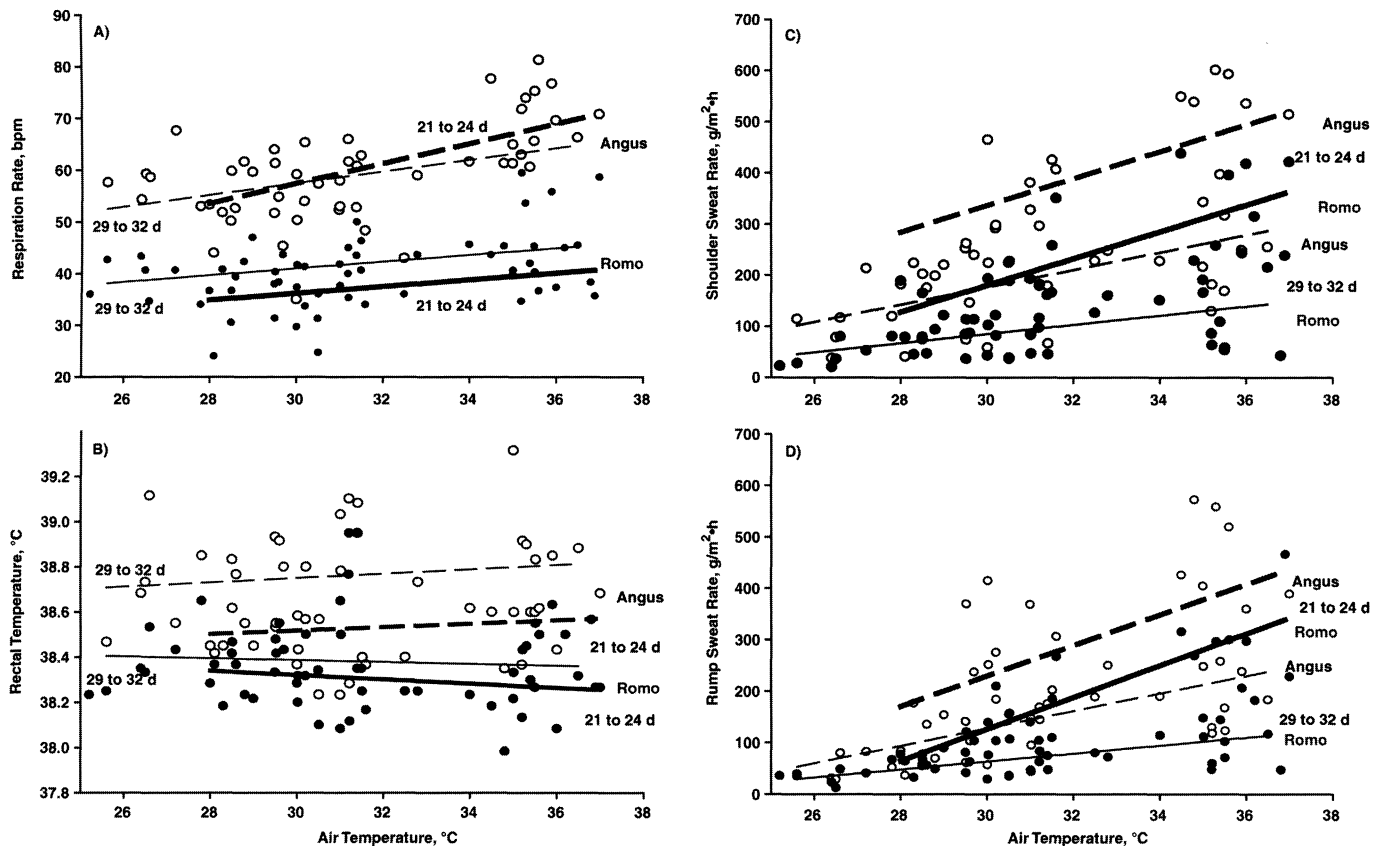
Figure 7. Heat stress-induced changes in respiration rate (A), rectal temperature (B), shoulder sweat rate (C), and rump sweat rate (D) are shown for Angus and Romosinuano (Romo) steers from early heat exposure (i.e., d 21 to 24) to late heat exposure (i.e., d 29 to 32). A linear fit is shown through points for each variable, breed, and set of days (air temperature: 26 to 36°C).

the greater rate ($P < 0.05$). Rectal temperature also showed no acclimation over the HS period, and neither breed exhibited a good correlation with T_a ($P > 0.10$; Figure 7B).

Blood Analyses

Several blood analyses, including urea nitrogen, potassium, creatinine phosphokinase, chloride, magnesium, glucose, and globulin, showed no breed or thermal effects (Table 2). Other blood variables, including albumin and triglyceride, revealed no breed differences, but exhibited HS-induced increases in both breeds ($P < 0.05$; Table 2). Throughout the study, breed differences were observed among cholesterol, creatinine, and leptin concentrations ($P < 0.05$). The most interesting of these responses were those that showed thermal × breed interactions. These variables included creatinine, cholesterol, prolactin, sodium, and total protein ($P < 0.05$; Table 2). Angus steers demonstrated HS-induced increases in prolactin, creatinine, and cholesterol ($P < 0.05$), but showed no breed differences at TN. There were also no breed differences ($P < 0.55$ and 0.16, respectively) between sodium and total protein levels. However, there were breed × time interactions for sodium and total protein with AG steers showing an increase in serum concentrations and RO showing a decrease ($P < 0.05$; Table 2).

DISCUSSION

Previous research has provided evidence that RO steers have a superior thermoregulatory ability compared with AG cattle (Spiers et al., 1994; Hammond et al., 1996). This ability must be the result of a reduction in heat production, increased capacity for heat loss to the environment, or a combination of both. There is ample evidence that basal metabolic rate of heat-tolerant *B. indicus* cattle is less than for heat-intolerant *B. taurus* cattle (Hansen, 2004). A reduced metabolic rate usually results in a reduced growth rate or reduced milk production (Hansen, 2004). Results from Chase et al. (1997) and Riley et al. (2007) have demonstrated that RO steers have a slower growth rate than other *B. taurus* breeds, again indicating that reduced metabolic rate may be a contributing factor to their thermotolerance. Steers in the present study were limit-fed and metabolic heat production was not measured; therefore, we did not quantify growth rate or feed intake differences. However, it has been documented that feed-restricted steers show a reduced rectal temperature (0.3 to 0.5°C) under hot environmental conditions (Davis et al., 2003). Because of this, animals from the current study might have been slightly less susceptible to the heat challenge. However, heat strain was demonstrated in both breeds used in this study, which allowed us to address the initial objective of determining if superior

Table 2. Breed averaged blood values during thermoneutral (TN; d 16; T_a: 19 to 21°C) and heat stress (HS; d 31; T_a: 26 to 36°C) periods with ±1 SE[1]

Item	Breed	TN	HS	±SE	P-value Breed	P-value Time	P-value Interaction
Albumin, g/dL	AG	3.15	3.45	0.04	0.47	0.001	0.12
	RO	3.28	3.44				
Chloride, mEq/L	AG	101.78	101.56	1.57	0.13	0.22	0.28
	RO	101.28	97.78				
Cholesterol, mg/dL	AG	64.11	91.55	6.17	0.01	0.001	0.001
	RO	58.01	64.88				
CPK, units/L	AG	126.44	114.44	20.90	0.80	0.53	0.98
	RO	131.00	118.00				
Creatinine, mg/dL	AG	1.16	1.51	0.07	0.01	0.01	0.05
	RO	1.07	1.11				
Globulin, g/dL	AG	3.08	3.21	0.14	0.90	0.75	0.12
	RO	3.26	3.06				
Glucose, mg/dL	AG	77.67	71.78	4.58	0.89	0.26	0.74
	RO	78.13	73.44				
Leptin, ng/mL	AG	4.45	5.47	0.38	0.01	0.05	0.56
	RO	3.63	4.22				
Magnesium, mg/dL	AG	2.24	2.41	0.06	0.84	0.06	0.32
	RO	2.31	2.36				
Potassium, mEq/L	AG	4.31	4.50	0.12	0.28	0.48	0.20
	RO	4.27	4.22				
Prolactin, ng/mL	AG	34.11	42.39	1.59	0.10	0.01	0.05
	RO	34.47	36.46				
Sodium, mg/dL	AG	141.11	144.44	1.31	0.55	0.95	0.05
	RO	143.63	140.44				
Total protein, g/dL	AG	6.23	6.67	0.16	0.66	0.08	0.05
	RO	6.55	6.51				
Triglyceride, mg/dL	AG	15.22	19.22	1.88	0.80	0.05	0.94
	RO	15.64	19.88				
Urea N, mg/dL	AG	7.00	6.89	0.67	0.08	0.25	0.32
	RO	6.38	4.89				

[1]T_a = air temperature; AG = Angus; RO = Romosinuano. CPK = creatine phosphokinase.

heat dissipation contributes to the underlying mechanism for heat tolerance of RO steers.

The most commonly used variable to assess heat tolerance is T_{re}. Because T_{re} is easy to measure, well documented in the literature, and heritability is moderate to low (i.e., 0.25, Turner, 1982; 0.33, Turner, 1984), it makes a reliable index. In a study by Hammond et al. (1996), AG heifers were contrasted with Brahman, RO, and Senepol heifers. Results showed that T_{re} in AG was greater than the 3 other breeds on the hottest day of summer (~1.0°C). Even when T_a was much cooler in the winter, T_{re} of the AG remained greater than in RO or Senepol breeds, although the differences in winter were no greater than 0.5°C (Hammond et al., 1996). These results are consistent with those found in the present study. Whether under TN conditions or during HS, the T_{re} of AG cattle was maintained at 0.5°C greater than RO cattle, with the exception of the first 3 d of heat. During the first few days after initiation of heat, T_{re} of both breeds overlapped before the T_{re} of RO returned to ~0.5°C less than AG. This is a common phenomenon during acute HS, with animals having to increase heat loss mechanisms to dissipate heat, followed by a reduction in feed intake and a reduction in metabolic heat production. Alterations in heat dissipation can occur

rapidly, whereas a change in metabolism takes longer. Typically, 3 to 4 d are necessary for an animal to begin acclimation and for rectal temperatures to be reduced to a new set point (Hahn, 1999). Even though they possessed the greater T_{re}, AG cattle maintained an ability to regulate core body temperature below 40°C.

Use of rectal or core temperature is not the only assessment of heat tolerance; respiratory dynamics are also a reliable indicator (Gaughan et al., 2009). It is known that RR or panting in cattle varies among individuals within a single breed (Bianca, 1963). However, RR during heat exposure is known to increase more rapidly than other responses and often occurs at a lesser critical T_a than other responses such as T_{re} or changes in feed intake (Hahn, 1999). In addition, RR (i.e., an indicator of respiratory evaporative heat loss) is one of several effector responses, including sweat rate and peripheral vasodilation, that determine the internal body temperature response to HS. In theory, it is only when the avenues for heat loss are compromised, or limits of effectiveness are reached, that there would be an increase in internal body temperature.

Respiration rates in the present study for AG during wk 1 at TN were increased above normal, which is likely due to a change in environment or other stressors.

However, by the end of wk 1, RR decreased to a similar level as others have reported at TN (Gaughan et al., 1999; Brown-Brandl et al., 2003; Beatty et al., 2006). The RO steers showed no such reduction, consistent with the breed being known as a docile breed (Chase et al., 1997). They maintained a RR less than AG steers and similar to what others have reported for Brahman steers (Hammond et al., 1996; Gaughan et al., 1999; Beatty et al., 2006).

Both breeds in the present study increased RR during heat exposure. Similarly to TN conditions, AG cattle had a greater RR under HS conditions than the RO cattle. This is consistent with the results reported in other studies (Spiers et al., 1994; Hammond et al., 1996). It is known that B. indicus breeds, specifically Brahman, have less RR compared with most B. taurus cattle. It is thought that heat-tolerant cattle rely on several physiological mechanisms to improve heat dissipation. Because the appearance of RO cattle and their heat tolerance ability are similar to Brahman cattle, they may have other characteristics in common. Most of these differences allow for greater heat dissipation through the skin, including greater blood flow to the skin and shorter hair coats (Finch, 1986). This could offer some explanation for the differences in RR between the 2 breeds in this study.

All steers in the present study showed a linear increase in T_{skin} with T_a. However, in RO steers, which maintained a lesser T_{skin} than AG steers at TN, the T_{skin} was not different from AG steers during HS. This phenomenon has been reported by others for B. indicus-type cattle. Allen (1962) compared Brahman and Jersey cattle T_{skin} at T_a from 24 to 35°C. He reported that B. indicus-type cattle had the lesser T_{skin} below T_a 24°C and the greater mean T_{skin} above T_a 35°C. An increase in the T_{skin} to T_a gradient enhances heat loss by radiation, conduction, and convection. Therefore, the increase in T_{skin} in the present study is advantageous to both breeds, as long as T_{skin} is below core temperature to maintain the outward flow of heat. The decreased T_{skin} of RO at TN suggests that the TN T_a represented a cool temperature for the RO steers, which would result in vasoconstriction.

Only 1 known study has examined sweating rate of RO cattle (Spiers et al., 1994). This study found that RO cattle exhibited a superior ability to sweat compared with AG cattle. Finch (1985) noted that when internal body temperature increases, sweating rate is greater and increases more rapidly in tropically adapted cattle than in temperate-zone B. taurus cattle. Therefore, expectations in the present study were that RO cattle would have a greater sweating rate than AG cattle. Surprisingly, results of the present study contradicted the results of Finch (1985) and Spiers et al. (1994). However, there are some major differences between the studies that may explain these results. Spiers et al. (1994) only measured sweating rate twice during their experiment. Spiers et al. (1994) also placed the animals in a constant HS compared with the cyclic method used in the present experiment. Although not many researchers have looked at sweat rate in heat-tolerant B. taurus cattle, research with B. indicus cattle has been conducted since the 1930s. Bos indicus cattle have been shown to have reduced sweating rates compared with B. taurus cattle under mild conditions, but greater rates at greater stress under acute conditions (Rhoad, 1940; Yeck and Kibler, 1956; Allen et al., 1963). With no nighttime cooling in the study conducted by Spiers et al. (1994), it is fair to say the cattle may have been under a greater heat strain. Variations in factors such as diet, coat cover, and acclimatization complicate comparisons and may also be responsible for some of the noted differences.

Another surprising result of the present study was a reduction in sweating rate of animals of both breeds after 7 d of cyclic HS. Studies measuring sweat rate have concentrated on either short-term controlled studies or seasonal differences in sweating (Allen, 1962; McLean, 1963; Schleger and Turner, 1965; Robertshaw and Taylor, 1969). This reduction has never been reported in cattle. A reduction in sweat rate after several hours of heat strain has been reported in sheep, goats, and humans (Collins and Weiner, 1962; Jenkinson et al., 1971). However, this reduction occurs when sweat rate is continuously monitored for secretion rates over minutes. This phenomenon, known as sweat gland fatigue, is believed to be due to the rate of expulsion exceeding the rate of sweat production (Jenkinson et al., 1971; Johnson, 1973). Although this may play a role in the reduction of sweat rate, it is unclear why steers from both breeds would not utilize this mechanism to regulate core body temperature. Interestingly, this reduction in sweat rate coincided with the increase in maximum T_{re} for AG steers beginning on d 10 of HS. Unexpectedly, RO cattle also maintained a reduced sweat rate during the second week of HS, but demonstrated no change in T_{re} at this time. It is unclear why this late increase in T_{re} occurred. Finch (1986) reported that a widening of the daily body temperature cycle in cattle arises from changes in energy and water metabolism. There is a possibility that water or mineral balance may have played a role in this delayed increase in core body temperature of AG cattle. Because water was available ad libitum, it is unlikely that water balance was a contributor.

In the present study, T_a showed the greatest correlation with all measured animal variables. These results are consistent with others (Scharf et al., 2008a), demonstrating that T_{skin} and RR exhibit good correlations with T_a, whereas T_{re} (i.e., the end result of thermoregulatory mechanisms) shows a poor correlation. Shoulder sweat rate vs. T_a and T_{skin} showed that T_a had a slightly greater correlation. The relationships between sweat rate and T_a and T_{skin} show these thermal variables could be the driving stimuli for sweating in cattle. The driving stimulus of sudomotor activity is

currently unknown (Scharf et al., 2008a). Interestingly, AG steers showed greater correlation coefficients across the board. This again illustrates that RO steers may have a superior ability to regulate internal body temperature than AG steers.

Several blood variables were measured in the present study to identify potential markers of heat sensitivity in cattle. Several of the measured blood variables did not change with HS and were not different between breeds. These included urea nitrogen, creatinine phosphokinase, magnesium, and chloride. Urea nitrogen can be indicative of dehydration (Schmidt-Nielsen and Schmidt-Nielsen, 1952) and protein catabolism (Srikandakumar et al., 2003). Creatinine phosphokinase is indicative of muscle injury or other pathologies (Spears et al., 1986). No change in these variables in the present study suggests that these conditions (i.e., muscle pathology, energy balance, or dehydration) did not exist. Both magnesium and chloride blood concentrations remained stable throughout the experiment. Because both breeds were fed a maintenance diet, the results demonstrated that the concentration of magnesium and chloride in the diet met the necessary requirements for the animal. Blood glucose concentrations also were not different between breeds or with HS, lending additional support for caloric balance in the present study.

Total protein represents a portion of the AA pool of the body and is believed to be indicative of the nutritional status of the animal (Doornenbal et al., 1988). Total protein consists mostly of albumin and globulin. In the present study, total blood protein content increased with HS, which has been reported by others (Shaffer et al., 1981). However, albumin showed an HS-related increase in both breeds, whereas globulin did not, indicating that the increase in total protein was primarily due to an increase in albumin. Like many blood variables, albumin is not well studied with regard to HS, but may be involved in some component of water balance (Parker et al., 2003).

No breed or HS differences were seen in serum sodium concentrations. However, there was a time × breed interaction with RO steers having a reduction in sodium concentration, whereas AG steers showed an increase. A reduction in serum sodium concentration is expected during HS and has been documented by El-Nouty et al. (1980). This reduction may be due to an increase in urinary sodium excretion due to increased total urinary output or expanded blood volume due to an increase in water intake. Normally, an increase in serum sodium could signify that an animal is becoming dehydrated. However, it is unlikely that dehydration resulted in any of the heat-induced changes or breed differences in sodium. Although water intake and drinking behavior were not measured in the present study, animals had ad libitum access to water. A commonly used variable as an indicator of dehydration is hematocrit or packed cell volume. However, it has been shown that hematocrit is not a reliable indicator of

dehydration because it is highly variable (Parker et al., 2004; Scharf et al., 2008b). One rapid response to dehydration is a significant reduction in feed intake (Bianca et al., 1965; Olsson, 2005; Scharf et al., 2008b). Even though animals in the current study were limit-fed, dehydration should have resulted in some reduction in feed consumption. Another indication of dehydration is an increase in plasma urea nitrogen. Previous research has shown that water restriction increases N retention, increasing serum concentrations in camels, sheep, and cattle (Schmidt-Nielsen and Schmidt-Nielsen, 1952; Goodall and Kay, 1968; Utley et al., 1970). Serum urea N in the current study did not change, further supporting the conclusion that water restriction was not an issue in the present study.

Unlike serum sodium, serum potassium showed no changes. This is expected as potassium is well maintained throughout the body. Some studies, including El-Nouty et al. (1980), found that potassium concentrations were reduced in cows during prolonged HS. El-Nouty et al. (1980) suggested that reductions in serum potassium were due to loss of potassium in sweat. Because cattle possess apocrine glands, secretions from the skin of cattle can contain 4 to 5 times the amount of potassium compared with sodium (Johnson, 1970). Because the HS blood sample was taken during wk 2 of HS, sweat rate had already acclimated to a reduced amount. Therefore, it may be that the dietary potassium in this experiment was enough to offset the losses from the skin.

Very little is known about breed differences in serum leptin concentrations. In the present study, AG steers had greater serum leptin concentration compared with RO steers throughout the study. This is consistent with Thomas et al. (2002) in which Angus bulls showed greater concentration of serum leptin in comparison with Brangus and Brahman bulls of similar age. It has been shown that plasma leptin is strongly related to adipose cell size and number in cattle (Delavaud et al., 2002). Because RO steers in this study were lighter and leaner than AG steers, it may be that adipose cells played a role in the differences in leptin concentration. To our knowledge, no one has examined leptin in regard to HS. In the present study, leptin increased in both breeds with HS. However, leptin has been implicated in reducing feed intake and alteration of heat shock protein 70, which can alter the HS response (Figueiredo et al., 2007).

Similar to leptin, serum creatinine showed breed differences throughout the study. However, only AG steers showed heat-induced increases. Romosinuano steers began the experiment with reduced concentrations than AG steers and showed no increase in serum creatinine concentrations with time. Increases in plasma creatinine during HS have been documented in sheep and cattle (Koubkova et al., 2002; Srikandakumar et al., 2003). Heat stress increases peripheral vasodilation to increase heat loss and reduces blood flow to the internal

organs (Srikandakumar et al., 2003). The rate of excretion of creatinine is influenced by renal perfusion and glomerular filtration rate. A reduction in renal blood flow during HS might then raise plasma creatinine concentration. With a large increase in T_{skin} (i.e., blood flow to the skin) with HS and no increase in serum creatinine in the RO steers, it may be that they have a greater ability to regulate blood flow to internal organs during HS or provide may provide additional support to the idea that RO steers were not significantly heat stressed in the present study.

Cholesterol was similar between the 2 breeds at TN. However, serum cholesterol in AG steers showed an increase during HS, whereas it remained unchanged in RO steers. Although not extensively studied in cattle, it has been reported that cholesterol increases during HS (Brody, 1956). Shaffer et al. (1981) reported that circulating cholesterol is influenced by the degree of stress. This is consistent with the hypothesis that the RO steers were not heat stressed to the same level as AG steers. The results of the present study were interesting given that it has been reported that other heat-tolerant breeds have greater cholesterol levels than *B. taurus* breeds (O'Kelly, 1968; Olbrich et al., 1971). Olbrich et al. (1971) found that the mean serum cholesterol concentrations were greater for Zebu than Scotch Highland heifers. O'Kelly (1968) also found that cholesterol, phospholipid, and total lipid concentrations were all significantly greater in Zebu than British breeds. It is unclear why RO steers would maintain reduced serum cholesterol in comparison with AG steers throughout the study.

Changes in serum prolactin concentrations in response to increases in T_a have been extensively studied and are known to be positively correlated (Schams, 1972; Wetteman and Tucker, 1974; Head et al., 1976; Johnson, 1985). Although plasma prolactin is known to increase during thermal stress, the mechanism is not well understood (Johnson, 1985). In the present study, there were no breed differences in serum prolactin concentration at TN, which is consistent with other reports between breeds (Ohlson et al., 1981; Wettemann et al., 1982). Serum prolactin concentrations in the present study increased with HS for the AG steers. However, no increase was seen for RO steers. Wettemann et al. (1982) reported that concentrations of serum prolactin in *B. indicus* and *B. taurus* heifers that were acutely and chronically exposed to various T_a responded similarly, suggesting that the mechanisms responsible for the control of prolactin in serum are not different between breeds. If this assumption is correct, it would again provide evidence that the RO steers were not heat stressed to the extent of AG steers in the current study. Although it is not understood why prolactin increases with T_a, prolactin shows potential for being an indicator of heat tolerance.

Angus and RO steers were tested in TN and under HS environments to determine differences between heat-sensitive and heat-tolerant *B. taurus* breeds. In both environments, AG cattle exhibited a greater heat loss (greater RR and sweat rate and similar T_{skin}) than the heat-tolerant breed. Surprisingly, the AG also exhibited the greater heat load as indicated by T_{re}. Romosinuano steers appeared to be only minimally heat stressed, showing an increase in RR but only a moderate rise in T_{re}. Because not all heat loss mechanisms were measured in the present experiment, it is impossible to definitively state the reason for the decreased T_{re} in RO. From these results, decreased heat production or a greater ability to vasodilate are the most likely candidates for the superior heat tolerance in RO steers.

A final objective of this study was to identify additional markers of heat tolerance. Rectal temperature is still a good indicator of heat tolerance. This study identified prolactin, cholesterol, and creatinine as additional markers of HS that require further research. Using these other variables, in addition to T_{re}, could allow for improved identification of heat-tolerant animals. Once animals are identified, selection pressure on heat tolerance and growth rate can be applied to increase animal productivity.

LITERATURE CITED

Allen, T. E. 1962. Responses of Zebu, Jersey and Zebu × Jersey crossbred heifers to rising temperature, with particular reference to sweating. Aust. J. Agric. Res. 13:165–179.

Allen, T. E., Y. S. Pan, and R. Hayman. 1963. The effect of feeding on the evaporative heat loss and body temperature in Zebu and Jersey heifers. Aust. J. Agric. Res. 14:580–593.

Beatty, D. T., A. Barnes, E. Taylor, D. Pethick, M. McCarthy, and S. K. Maloney. 2006. Physiological responses of *Bos taurus* and *Bos indicus* cattle to prolonged, continuous heat and humidity. J. Anim. Sci. 84:972–985.

Bianca, W. 1963. Rectal temperature and respiratory rate as indicators of heat tolerance in cattle. J. Agric. Sci. 60:113–120.

Bianca, W., J. D. Findlay, and J. A. McLean. 1965. Responses of steers to water restriction. Res. Vet. Sci. 6:38–55.

Bligh, J., and K. G. Johnson. 1973. Glossary of terms for thermal physiology. J. Appl. Physiol. 35:941–961.

Brody, S. 1956. Climatic physiology of cattle. J. Dairy Sci. 39:715–725.

Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, and H. Freetly. 2003. Thermoregulatory responses of feeder cattle. J. Therm. Biol. 28:149–157.

Burton, A. C., and O. G. Edholm. 1955. Man in a Cold Environment. Edward Arnold Ltd., London, UK.

Chase, C. C., Jr., A. C. Hammond, T. A. Olson, C. N. Murphy, A. J. Tewolde, and L. Griffin. 1997. Introduction of Romosinuano in the U.S.A. Arch. Latinoam. Prod. Anim 5:57–71.

Collins, K. J., and J. S. Weiner. 1962. Observations on arm-bag suppression of sweating and its relationship to thermal sweat-gland 'fatigue'. J. Physiol. 161:538–556.

Davis, M. S., T. L. Mader, S. M. Holt, and A. M. Parkhurst. 2003. Strategies to reduce feedlot cattle heat stress: Effects on tympanic temperature. J. Anim. Sci. 81:649–661.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519–526.

Delavaud, C., A. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann, and Y. Chilliard. 2002. Plasma leptin concentration in adult cattle: Effects of breed, adiposity, feeding level and meal intake. J. Anim. Sci. 80:1317–1328.

Doornenbal, H., A. K. W. Tong, and N. L. Murray. 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. Can. J. Vet. Res. 52:99–105.

El-Nouty, F. D., I. M. Elbanna, T. P. Davis, and H. D. Johnson. 1980. Aldosterone and ADH response to heat and dehydration in cattle. J. Appl. Physiol. 48:249–255.

Elzo, M. A., C. Manrique, G. Ossa, and O. Acosta. 1998. Additive and nonadditive genetic variability for growth traits in the Turipaná Romosinuano-Zebu multibreed herd. J. Anim. Sci. 76:1539–1549.

Figueiredo, D., A. Gertler, G. Cabello, E. Decupere, J. Buyse, and S. Dridi. 2007. Leptin down regulates heat shock protein-70 (HSP-70) gene expression in chicken liver and hypothalamus. Cell Tissue Res. 329:91–101.

Finch, V. A. 1985. Comparison of non-evaporative heat transfer in different cattle breeds. Aust. J. Agric. Res. 36:497–508.

Finch, V. A. 1986. Body temperature in cattle: Its control and relevance to production in the tropics. J. Anim. Sci. 62:531–542.

Finch, V. A., I. L. Bennet, and C. R. Holmes. 1982. Sweating response in cattle and its relation to rectal temperature, tolerance of sun and metabolic rate. J. Agric. Sci. 99:479–487.

Gaughan, J. B., T. L. Mader, S. M. Holt, M. J. Josey, and K. J. Rowan. 1999. Heat tolerance of Boran and Tuli crossbred steers. J. Anim. Sci. 77:2398–2405.

Gaughan, J. B., T. L. Mader, S. M. Holt, M. L. Sullivan, and G. L. Hahn. 2009. Assessing the heat tolerance of 17 beef cattle genotypes. Int. J. Biometeorol. doi:10.1007/s00484-009-0233-4

Gebremedhin, K., P. Hillman, C. Lee, and R. Collier. 2007. Sweating rate of dairy cows under shade and sunny environments. 2007 ASAE Annual Meeting, Minneapolis, MN. Paper No. 074083.

Goodall, E. D., and R. N. B. Kay. 1968. Water intake and the cycling of nitrogen to the stomach in sheep. J. Physiol. 194:38P–39P.

Hahn, G. L. 1999. Dynamic responses of cattle to thermal heat loads. J. Anim. Sci. 77:10–20.

Hammond, A. C., C. C. Chase Jr., E. J. Bowers, T. A. Olson, and R. D. Randel. 1996. Heat tolerance in Tuli-, Senepol-, and Brahman-sired F_1 Angus heifers in Florida. J. Anim. Sci. 76:1568–1577.

Hansen, P. J. 2004. Physiological and cellular adaptations of Zebu cattle to thermal stress. Anim. Reprod. Sci. 82–83:349–360.

Head, H. H., W. W. Thatcher, C. J. Wilcox, and K. C. Bachman. 1976. Effect of a synthetic corticoid on milk yield and composition and on blood metabolites and hormones in dairy cows. J. Dairy Sci. 59:880–888.

Jenkinson, D. M. E., D. McEwan, and D. Robertshaw. 1971. Studies on the nature of sweat gland 'fatigue' in the goat. J. Physiol. 212:455–465.

Johnson, H. D. 1985. Physiological responses and productivity of cattle. Pages 3–24 in Volume 1: Stress Physiology in Livestock. Basic Principles. M. K. Yousef, ed. CRC Press, Boca Raton, FL.

Johnson, K. G. 1970. Sweating rate and the electrolyte content of skin secretions of *Bos taurus* and *Bos indicus* cross-bred cows. J. Agric. Sci. 75:397–402.

Johnson, K. G. 1973. Sweat storage as a factor influencing sweat discharge in sheep. J. Physiol. 235:523–534.

Koubkova, M., I. Knizkova, P. Kunc, H. Hartlova, J. Flusser, and O. Dolezal. 2002. Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. Czech J. Anim. Sci. 47:309–318.

Lutz, S. L., M. F. Smith, D. H. Keisler, and H. A. Garverick. 1991. Effect of constant infusion of oxytocin on luteal lifespan and oxytocin-induced release of prostaglandin $F_{2\alpha}$ in heifers. Domest. Anim. Endocrinol. 8:573–585.

McLean, J. A. 1963. The regional distribution of cutaneous moisture vaporization in the Ayrshire calf. J. Agric. Sci. Camb. 61:275–280.

Nuutinen, J., E. Alanen, P. Autio, M. Lahtinen, I. Harvima, and T. Lahtinen. 2003. A closed unventilated chamber for the measurement of transepidermal water loss. Skin Res. Technol. 9:85–89.

Ohlson, D. L., S. L. Davis, C. L. Ferrell, and T. G. Jenkins. 1981. Plasma growth hormone, prolactin and thyrotropic secretory patterns in Hereford and Simmental calves. J. Anim. Sci. 53:371–375.

O'Kelly, J. C. 1968. Comparative studies of lipid metabolism in Zebu and British cattle in a tropical environment. I. Plasma lipid levels of grazing cattle. Aust. J. Biol. Sci. 21:1013–1024.

Olbrich, S. E., F. A. Martz, M. E. Tumbleson, H. D. Johnson, and E. S. Hilderbrand. 1971. Serum biochemical and hematological measurements of heat tolerant (Zebu) and cold tolerant (Scotch Highland) heifers. J. Anim. Sci. 33:655–658.

Olsson, K. 2005. Fluid balance in ruminants: Adaptation to external and internal challenges. Ann. N. Y. Acad. Sci. 1040:156–161.

Parker, A. J., G. P. Hamlin, C. J. Coleman, and L. A. Fitzpatrick. 2003. Dehydration in stressed ruminants may be the result of acortisol-induced diuresis. J. Anim. Sci. 81:512–519.

Parker, A. J., G. P. Hamlin, C. J. Coleman, and L. A. Fitzpatrick. 2004. Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. J. Anim. Sci. 82:1037–1045.

Rhoad, A. O. 1940. Absorption and reflection of solar radiation in relation to coat color in cattle. Am. Soc. Anim. Prod. 1940:291–293.

Riley, D. G., C. C. Chase Jr., S. W. Coleman, and T. A. Olson. 2007. Evaluation of birth and weaning traits of Romosinuano calves as purebreds and crosses with Brahman and Angus. J. Anim. Sci. 85:289–298.

Robertshaw, D., and C. R. Taylor. 1969. A comparison of sweat gland activity in eight species of East African bovids. J. Physiol. 203:135–143.

Schams, D. 1972. Prolactin levels in bovine blood, influenced by milking manipulation, genital stimulation and oxytocin administration with specific consideration of the seasonal variations. Acta Endocrinol. (Copenh.) 71:684–696.

Scharf, B., L. E. Wax, G. E. Aiken, and D. E. Spiers. 2008a. Regional differences in sweat rate response of steers to short-term heat stress. Int. J. Biometeorol. 52:725–732.

Scharf, B., L. E. Wax, T. J. Evans, and D. E. Spiers. 2008b. Impact of dehydration on production and thermoregulation of Angus steers at thermoneutrality. Pages 465–471 in Proc. 8th Int. Livest. Environ. August 31 to September 4, 2008, Iguassu Falls, Brazil. Am. Soc. Agric. Biol. Eng., St. Joseph, MI.

Schleger, A. V., and H. G. Turner. 1965. Sweating rates of cattle in the field and their reaction to diurnal and seasonal changes. Aust. J. Agric. Res. 16:92–106.

Schmidt-Nielsen, K., and B. Schmidt-Nielsen. 1952. Water metabolism of desert mammals. Physiol. Rev. 32:135–166.

Shaffer, L., J. D. Roussel, and K. L. Koonce. 1981. Effects of age, temperature-season, and breed on blood characteristics of dairy cattle. J. Dairy Sci. 64:62–70.

Spears, J. W., R. W. Harvey, and E. C. Segerson. 1986. Effects of marginal selenium deficiency and winter protein supplementation on growth, reproduction and selenium status of beef cattle. J. Anim. Sci. 63:586–594.

Spiers, D. E., D. W. Vogt, H. D. Johnson, G. B. Garner, and C. N. Murphy. 1994. Heat-stress responses of temperate and tropical breeds of *Bos taurus* cattle. Arch. Latinoam. Prod. Anim. 2:41–52.

Srikandakumar, A., E. H. Johnson, and O. Mahgoub. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin. Res. 49:193–198.

Steele, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill Publishing Co, New York, NY.

Thomas, M. G., R. M. Enns, D. M. Hallford, D. H. Keisler, B. S. Obeidat, C. D. Morrison, J. A. Hernandez, W. D. Bryant, R. Flores, R. Lopez, and L. Narro. 2002. Relationships of meta-

bolic hormones and serum glucose to growth and reproductive development in performance-tested Angus, Brangus, and Brahman bulls. J. Anim. Sci. 80:757–767.

Turner, H. G. 1982. Genetic variation of rectal temperature in cows and its relationship to fertility. Anim. Prod. 35:401–412.

Turner, H. G. 1984. Variation in rectal temperature of cattle in a tropical environment and its relation to growth rate. Anim. Prod. 38:417–427.

Utley, P. R., N. W. Bradley, and J. A. Boling. 1970. Effect of restricted water intake on feed intake, nutrient digestibility and nitrogen metabolism in steers. J. Anim. Sci. 31:130–135.

Vogt, D. C., C. Murphy, R. Crawford, A. Clarke, E. Cole, G. Garner, and A. Tewolde. 1991. Comparison of growth, carcass and meat characteristics of straightbred Angus and Romo Sinuano × Angus cross cattle managed under a forage system. Mo. Agric. Exp. Sta. Southwest Center Research Report, Vernon, MO.

Wettemann, R. P., and H. A. Tucker. 1974. Relationship of ambient temperature to serum prolactin in heifers. Proc. Soc. Exp. Biol. Med. 146:908–911.

Wettemann, R. P., H. A. Tucker, T. W. Beck, and D. C. Meyerhoeffer. 1982. Influence of ambient temperature on prolactin concentrations in serum of Holstein and Brahman × Hereford heifers. J. Anim. Sci. 55:391–394.

Yeck, R. G., and H. H. Kibler. 1956. Moisture vaporization by Jersey and Holstein cows during diurnal temperature cycles as measured with a hygrometric tent. Mo. Agric. Exp. Sta. Res. Bull. 600.